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A Hairpin Turn in a HIV-gag–Derived Peptide Bound to HLA-DR1 Orients Peptide Residues Outside the Binding Groove for T Cell Recognition

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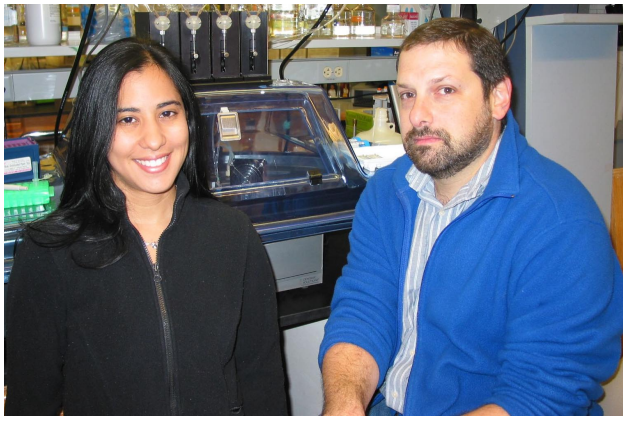
T-cell receptors (TCRs) recognize peptide antigens bound to class II Major Histocompatibility Complex (MHC) proteins—molecules that transport and present the antigens to the T-cells, thus activating the immune system response. The receptors contact the antigen residues within or immediately flanking the seven-to-nine-residue sequence accommodated by the MHC-peptide binding groove. We identified a HIV-Gag-specific T-cell clone that requires an unusually long peptide for activation. The crystal structure of the HIV-gag peptide bound to an MHC protein known as HLA-DR1 shows that the peptide binds in an unexpected conformation, with its C-terminal region making a hairpin turn that bends back over the groove. The residues at the C-terminus are critical for T-cell recognition, and disrupting the hairpin cancels the immune response. The results suggest a new mode of MHC-peptide-TCR interaction.

Major Histocompatibility Complex (MHC) proteins are heterodimeric cell surface proteins that present antigens to T-cells, triggering the cell-mediated immune system. Peptides isolated from class II MHCs found in antigen-presenting cells are usually 15-20 residues long. Approximately one-third of the total peptide surface (the central region of the peptide) is accessible to solvent, which is necessary for recognition by the T-cell receptors (TCR). Typically, TCRs specifically recognize a stretch of approximately nine residues.

We investigated the binding of peptides to class II MHC molecules using x-ray crystallography. The structures show that peptides bind in a polyproline type-II conformation with several conserved binding pockets within the overall MHC peptide-binding groove. Generally, the pockets accommodate the side chains of peptide residues at the P1, P4, P6, and P9 positions, with smaller pockets or shelves for the P3 and P7 residues. In the canonical conformation, the side chains of residues at the P-1, P2, P5, and P8 locations are solvent-accessible and point toward the TCRs. Mutagenesis studies of many MHC-TCR pairs, in addition to the two available MHC-peptide-TCR crystal structures, show that the TCRs contact the MHC-bound peptide in the region circumscribed by MHC-peptide contacts at the P-1, P2, P3, P5, and P8 positions.

A T-cell clone isolated from an individual acutely infected with HIV-1 recognizes a peptide antigen derived from the HIV-Gag protein bound to the human class II MHC protein HLA-DR1. This clone requires an unusually long version of the peptide for activation.



Dr. Zarixia Zavala-Ruiz (left) and Prof. Lawrence J. Stern.

The crystal structure of HLA-DR1 in complex with the unusually long Gag-derived peptide, Gag[PP16], shows that the peptide binds in an unexpected bent conformation, forming a hairpin turn that orients the C-terminal region above the remainder of the peptide. An alignment of HLA-DR1-Gag[PP16] with other crystal structures of HLA-DR complexes for which clear electron density can be seen beyond the P10 peptide residue is shown in **Figure 1**. On each of the other HLA-DR complex structures, the peptide extends straight out of the binding site.

To determine the importance of the C-terminal region in T-cell activation and to confirm the physiological relevance of the hairpin observed in the

crystal structure, we performed alanine scanning mutagenesis. Each Gag[PP16] peptide residue was changed independently to alanine and standard MHC-peptide binding assays and T-cell activation experiments were conducted. In the MHC-peptide binding assay, we saw some significant reductions in binding affinity upon alanine substitution of Val(P1) and Met(P4) and Ser(P9). These effects are consistent with the binding frame observed in the crystal structure. In the T-cell activation assay, alanine substitutions of Glu(P-1), Ile(P2), Phe(P5) and Thr(P13) completely abolished T-cell activation. The side chains located at positions P-1, P2, and P5 are solvent-exposed and therefore accessible for recognition by the TCRs (**Figure 2**). Substitution of Gly(P11) to proline, which cannot be accommodated in the hairpin, also abolished T-cell activation.

In conclusion, our study shows that peptides can bind to MHC II in previously unobserved conformations in which residues outside the binding groove can bend back and become accessible for TCR recognition. This provides evidence for a new mode for MHC-peptide-TCR interaction.

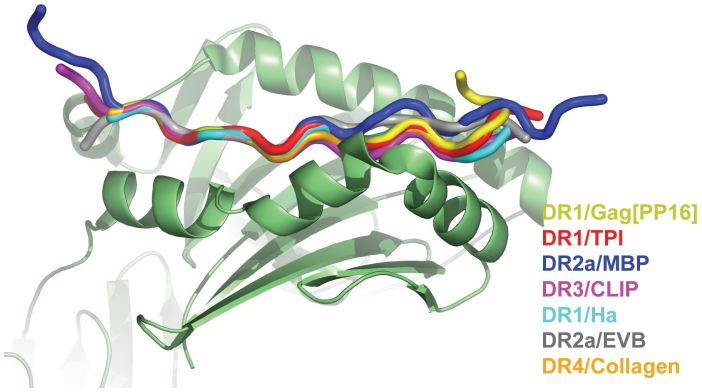


Figure 1. *Alignment of HLA-DR crystal structure exhibiting ordered peptide density beyond the P10 residue. Complexes were aligned by least-squares fitting of α1 and β1 domains of the MHC protein. Peptides are shown as Cα traces. DR1/TPI is red (PDB ID code 1KLU), DR2a/MBP is blue (PDB ID code 1FV1), DR3/CLIP is magenta (PDB ID code 1A6A), DR1/Ha is cyan (PDB ID code 1DLH), DR2a/EBV is gray (PDB ID code 1H15), and DR4/collagen is orange (PDB ID code 2SEB).*

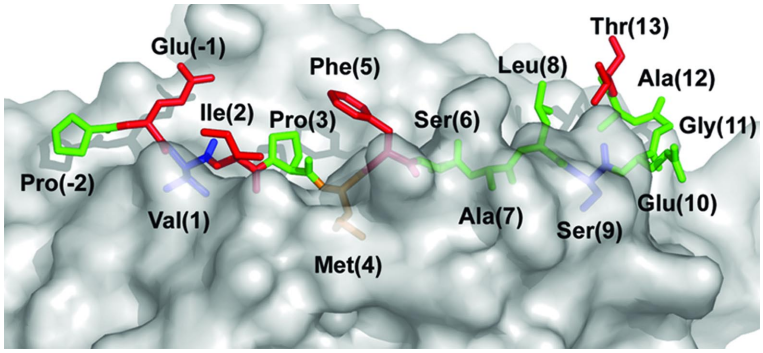


Figure 2. *The Gag[PP16] hairpin turns orients important C-terminal residues in position to interact with the TCR. Side view of surface of HLA-DR1 with the Gag[PP16] peptide shown as a stick model. Peptide side chains essential for T-cell activation are red and those important for peptide binding to HLA-DR1 are blue. Met(P4) is in orange, it affects both MHC peptide binding and T-cell activation*